IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 7947
William Pollack	Examiner: V. Ford
Application No.: 09/660,862	Technology Center/Art Unit: 1645
Filed: September 13, 2000	PRELIMINARY AMENDMENT
For: METHOD OF MANUFACTURING IMMUNE GLOBULIN	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, William Pollack, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently chairman and chief executive officer of Atopix Pharmaceuticals Corporation, the assignee of the subject application.

3. I, Dr. Pollack, graduated from the Imperial College of Science and Technology at London University with a B.Sc degree in chemistry and physics. I received a M.Sc degree in physiology and biochemistry from St. Georges Hospital Medical School at London University and a Ph.D. in immunology and immunochemistry from Rutgers University. A copy of my curriculum vitae is attached hereto as Exhibit A.

4. I am the named and true inventor of the above-referenced patent application. I have read and am familiar with the contents of the patent application. In addition, I have read the final Office Action, dated September 8, 2004, received in the present case. It is

my understanding that the Examiner believes that the Laursen *et al.* patent, U.S. Patent Number 6,281,336, in combination with Flaa *et al.*, patent, U.S. Patent Number 6,165,981 makes the invention of the present application obvious.

5. With this application, I claim a method of manufacturing a highly purified IgG4 immune globulin preparation. The method comprises the steps of adjusting plasma to a pH of 6.5 and a conductivity of between 3.5-6 millisiemens, and contacting the plasma with an anion exchange resin followed by a cation exchange resin to separate the IgG4 from other IgG subtypes and obtain a final effluent that comprises IgG4 essentially free of other IgG subtypes.

6. The Laursen *et al.* method, unlike the method of the present application, does not result in an immunoglobulin preparation comprising IgG4 that is essentially free of other IgG subtypes. The Laursen *et al.* method, instead, results in a purified stable IgG gamma globulin preparation (also referred to as IVIG) containing all IgG subtypes including IgG1, IgG2, IgG3, and IgG4. Laursen *et al* does not suggest or teach separation of IgG4 from other IgG subtypes to arrive at an IgG4 that is essentially free of other IGg subtypes. Laursen *et al.* neither suggest nor teach that their purification method results in pure IgG4 essentially free of all other subtypes, nor, in my opinion, could the purification system described in Laursen *et al.* result in IgG4 essentially free of other IgG subtypes.

7. The Office Action asserts that Laursen *et al.* teach a method of producing IgG4. This assertion appears to be based on a table showing IgG subtype distribution of total IgG preparations and a description of how the values in the table were determined. See, *e.g.*, Laursen *et al.*, Table at columns 17 and 18, and column 20, lines 9-15. In my opinion, the passages cited by the Office Action do not demonstrate the claimed production of IgG4 that is essentially free of other IgG subtypes and do not demonstrate methods of separating IgG4 from other IgG subtypes to produce IgG4 that is essentially free of other IgG subtypes.

8. Laursen *et al.* disclose methods to purify total IgG from other plasma components. Laursen *et al.* teach adjustment of plasma protein pH to a pH less than 6.0, preferably pH 5.4. The total IgG proteins are solubilized using PEG precipitation which also removes viral particles. The soluble IgG proteins are than subjected to anion exchange chromatography followed by cation exchange chromatography. Laursen *et al.* teach that the total

IgG fraction binds to the cation exchange resin at a pH less than pH 6.0. A salt gradient us used to displace the total IgG fraction, including IgG4 from the cation exchange resin. See, *e.g.*, Laursen *et al* at column 6, lines 61-64; column 8, lines 32-37 and 45-60. The cited Table at columns 17 and 18 shows the IgG subtype distribution found in the purified total IgG preparation made by the above-described methods of Laursen *et al.* for comparison to commercially available IgG products. The Laursen *et al.* total IgG product includes 98.5% IgG1, IgG2, or IgG3 subtypes and only 1.5% IgG4 subtype. Thus, the disclosure of Laursen *et al.* do not teach methods of separating IgG4 from other IgG subtypes to produce IgG4 that is essentially free of other IgG subtypes.

9. Laursen *et al.* disclosed the subtype distribution of their total IgG product in the Table at columns 17 and 18. Laursen et al. determined the subtype distribution using a quantitative method called Mancini immunodiffusion. See, *e.g.*, Laursen *et al.*, at column 20, lines 6-15. Mancini immunodiffusion is a semi-quantitative technique used for quantitative analysis of plasma proteins and in my opinion, is of no value for the fractional separation of proteins, particularly immunoglobulins from one another. In fact, as performed, the assay adds additional IgG antibodies to the total IgG sample of Laursen *et al.* solely for quantification purposes. A brief description of the Mancini immunodiffusion technique follows. To determine the subtype distribution within the total IgG preparation, a sample of the total IgG preparation is added to agar containing an antibody specific for one of the four IgG subtypes (an anti-IgG subtype antibody). If an IgG subtype is recognized by the antibody in the agar, the IgG subtype binds to the antibody forming an IgG subtype-antibody-complex demonstrated as a visible turbid ring of precipitation in the agar. The area of precipitation is proportional to concentration of the IgG subtype, and thus the amount IgG subtypes within the total IgG sample can be determined. The specific IgG subtypes do not require separation from other components of the total IgG sample for formation of the detectable IgG subtype-antibody-complex and, therefore, separations of individual IgG subtypes are not performed. The end product of a Mancini immunodiffusion assay is an agar gel containing a mixture of unbound IgG subtypes from the original sample, unbound specific antibody used for detection and an IgG subtype-anti-IgG antibody complex. For example, if the assay was used to determine a level of IgG4 in the total IgG sample, the end product would be agar containing a mixture of unbound IgG1, 2, 3 and 4 proteins, unbound anti-

IgG4 antibody and IgG4 antigen-antibody complexes. A schematic drawing showing a Mancini immunodiffusion assay is included as Exhibit B. It should be recognized that for the assay described in Laursen *et al.* the antigen is the total IgG preparation and thus, a mixture of at least four types of IgG proteins. Moreover, an IgG4-anti-IgG4 complex is not IgG4 essentially free of other IgG subtypes because the IgG1, 2 and 3 subtypes are also present within the agar matrix. Thus, while the assay can be used to determine a level of a particular IgG subtype, such as IgG4, the assay does not provide a method of separating IgG4 from other IgG subtypes to produce IgG4 that is essentially free of other IgG subtypes.

10. The Office Action appears to assert that modification of Laursen *et al.* to arrive at the claimed method of manufacture of IgG4 that is essentially free of other IgG subtypes is obvious. In my opinion, the modification of the methods of Laursen *et al.* suggested by the Office Action would render the both the methods and the disclosed total IgG product of Laursen *et al.* unsuitable for its intended purpose.

11. Laursen *et al.* disclose manufacture of total IgG with the intent to administer the product to patients that require treatment of diseases or conditions that benefit from replacement or supplementation of the total IgG component of blood, including *e.g.*, primary and secondary agammaglobulinemia, Wiskott-Aldrich syndrome, severe combined immunodeficiency, treatment of autoimmune diseases, and treatment of certain patients with immune conditions. See, e.g., Laursen *et al.* at column 15, lines 8-47. A purified IgG4 preparation would not be useful to treat disease that require supplementation of total IgG. Therefore, in my opinion, modification of the methods of Laursen *et al.* to arrive at the claimed methods to manufacture IgG4 that is essentially free of other IgG subtypes would render the methods of Laursen *et al.* unfit for their intended purpose of manufacturing a total IgG product for treatment of diseases or conditions that benefit from replacement or supplementation of the total IgG component of blood.

12. Laursen *et al.* specifically disclaim adjustment of plasma pH to values greater than pH 6.0. See, e.g., Laursen *et al.*, at column 5, lines 12-17. A pH below 6.0 is required to optimize a subsequent PEG precipitation step. The PEG precipitation step is used to stabilize the total IgG fraction, while precipitating out infectious viral particles. Laursen *et al.* at column 6, lines 1-6, increasing the pH of plasma as specifically disclaimed by Laursen *et al.*

would most likely decrease the effectiveness of the PEG precipitation step by *e.g.*, decreasing the stability of the IgG and/or reducing the effectiveness of virus particle removal. Either result, in my opinion, would render the Laursen *et al.* methods unsuitable for production of a stable, virus-free, purified total IgG component of blood for safe administration to patients with diseases or conditions that benefit from replacement or supplementation of the total IgG component of blood and thus, unsuitable for intended purpose of the methods.

13. Further, Larsen *et al.* in view of Flaa *et al.* does not make obvious the manufacturing method of the present application, as the secondary reference of Flaa *et al.* does not address the deficiencies in the Larsen *et al.*

14. Flaa *et al.* teach only solutions for stabilizing purified proteins and do not teach any methods for separating proteins, including immunoglobulins, and in particular IgG subtypes such as IgG4.

15. In view of the foregoing, it is my scientific opinion that, after reading the above mentioned references, the presently claimed method is novel and unobvious over the cited art. Based on the disclosure of the cited references, one of skill in the art would not be motivated to make the purified IgG4 preparations using the method of the present application. Therefore, Laursen *et al.* either alone or in combination with Flaa *et al.* does not make the invention of the present application obvious.

The declarant has further nothing to say.

Date: 12-22-04 By: William Pollack

William Pollack, Ph.D.

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CURRICULUM VITAE
OF
DR. WILLIAM POLLACK

Education

Imperial College of Science & Technology. (London University, England).
B.Sc., A.R.C.S., (Major Chemistry & Physics.)

St. George's Hospital Medical School. (London University. England). M.Sc., (Physiology & Biochemistry). F.R.C.Path.

Rutgers - the State University of New Jersey.
Ph.D. (Immunology & Immunochemistry). Dissertation thesis: *"A study of the Factors Affecting the Zeta-Potential and Hemagglutination with Human Iso-Antibodies".*

Professional History

1994-Present	Atopix Pharmaceuticals Corporation Carlsbad, California, 92008 Chairman & C.E.O.
1981-1985	The Purdue Frederick Co. Norfolk, Conn. 06856 Vice President, R/D Member of Executive Committee.
1956-1981	Ortho Diagnostics Systems, Inc. Raritan, NJ 08869 (A Johnson & Johnson Co.) Vice President and Member of Board of Directors Director of Research.
1954-1956	Royal Columbian Hospital British Columbia, Canada. Director of Blood Bank & Clinical Laboratories.
1948-1954	St. George's Hospital Medical School, London, England
1943-1946	Royal Navy, Honorable Discharge Lieutenant, Service in North Atlantic and Pacific

Academic Appointments

1974-1985	Associate Adjunct Professor. University of Medicine & Dentistry of New Jersey. (Previously Rutgers Medical School)
1968-1981	Associate Clinical Professor of Pathology College of Physicians and Surgeons Columbia University, New York City, NY.

Awards and Honors

1969	Karl Landsteiner Award of the American Association of Blood Banks.
1976	John Scott Award, Philadelphia Board of Directors of City Trusts.
1978	XXXI Annual Gibson Lecturer, Columbia Presbyterian Medical Center, New York City.
1979	Joseph Bolivar-DeLee Humanitarian Award. Chicago, Illinois.
1980	Albert and Mary Lasker Clinical Medical Research Award.
1987	Award from New York State Perinatal Society for Unique Contributions to Maternal & Child Health.
	Listed in: Who's Who in America, Who's Who in the World and American Men and Women of Science.

Present and Past Professional and Honorary Societies

American Association for the Advancement of Science
American Association of Blood Banks
American Association of Immunologists
American Society of Clinical Pathologists
American Society of Hematology
British Society of Immunology
Harvey Society
International Society of Blood Transfusion
New York Academy of Medicine
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Reticuloendothelial Society
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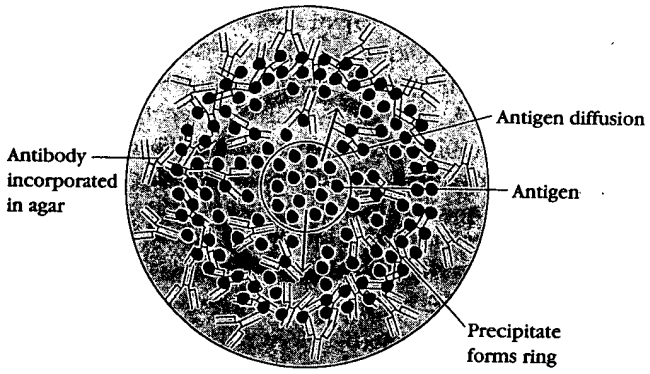
Precipitation Reactions in Gels

Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward one another in agar or when antibody is incorporated into the agar and antigen diffuses into the antibody-containing matrix, a visible line of precipitation will form. As in a precipitation reaction in fluid, visible precipitation occurs in the region of equivalence, whereas no visible precipitate forms in regions of antibody or antigen excess. These **immunodiffusion reactions** can be used to determine relative concentrations of antibodies or antigens, to compare antigens, or to determine the relative purity of an antigen preparation. Two frequently used immunodiffusion techniques are **radial immunodiffusion** (Mancini method) and **double immunodiffusion** (Ouchterlony method); both are carried out in a semisolid medium like agar (Figure 6-6).

RADIAL IMMUNODIFFUSION



DOUBLE IMMUNODIFFUSION

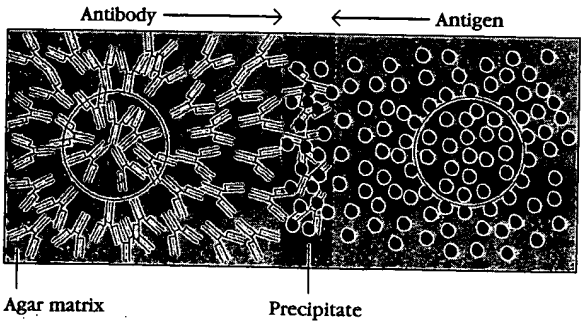


FIGURE 6-6

Diagrammatic representation of radial (Mancini) and double immunodiffusion (Ouchterlony) in a gel. In both cases, large insoluble complexes form in the agar in the zone of equivalence, which are visible as a line of precipitation (blue region). Only the antigen diffuses in radial immunodiffusion, whereas both the antibody and antigen diffuse in double immunodiffusion.

Radial Immunodiffusion (Mancini Method)

The relative concentrations of an antigen can be determined by a simple quantitative assay in which an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum. As the antigen diffuses into the agar, the region of equivalence is established and a ring of precipitation forms around the well (see Figure 6-6). The area of the precipitin ring is proportional to the concentration of antigen. By comparing the area of the precipitin ring with a standard curve (obtained by measuring the precipitin areas of known concentrations of the antigen), the concentration of the antigen sample can be determined.

The Mancini method is routinely used to quantitate serum levels of IgM, IgG, and IgA by incorporating class-specific anti-isotype antibody into the agar (Figure 6-7). The technique is also applied to determine the concentrations of complement components in serum. The Mancini method cannot detect antigens present in concentrations below 5–10 μg/ml; this moderate sensitivity is the major limitation of the radial immunodiffusion method.

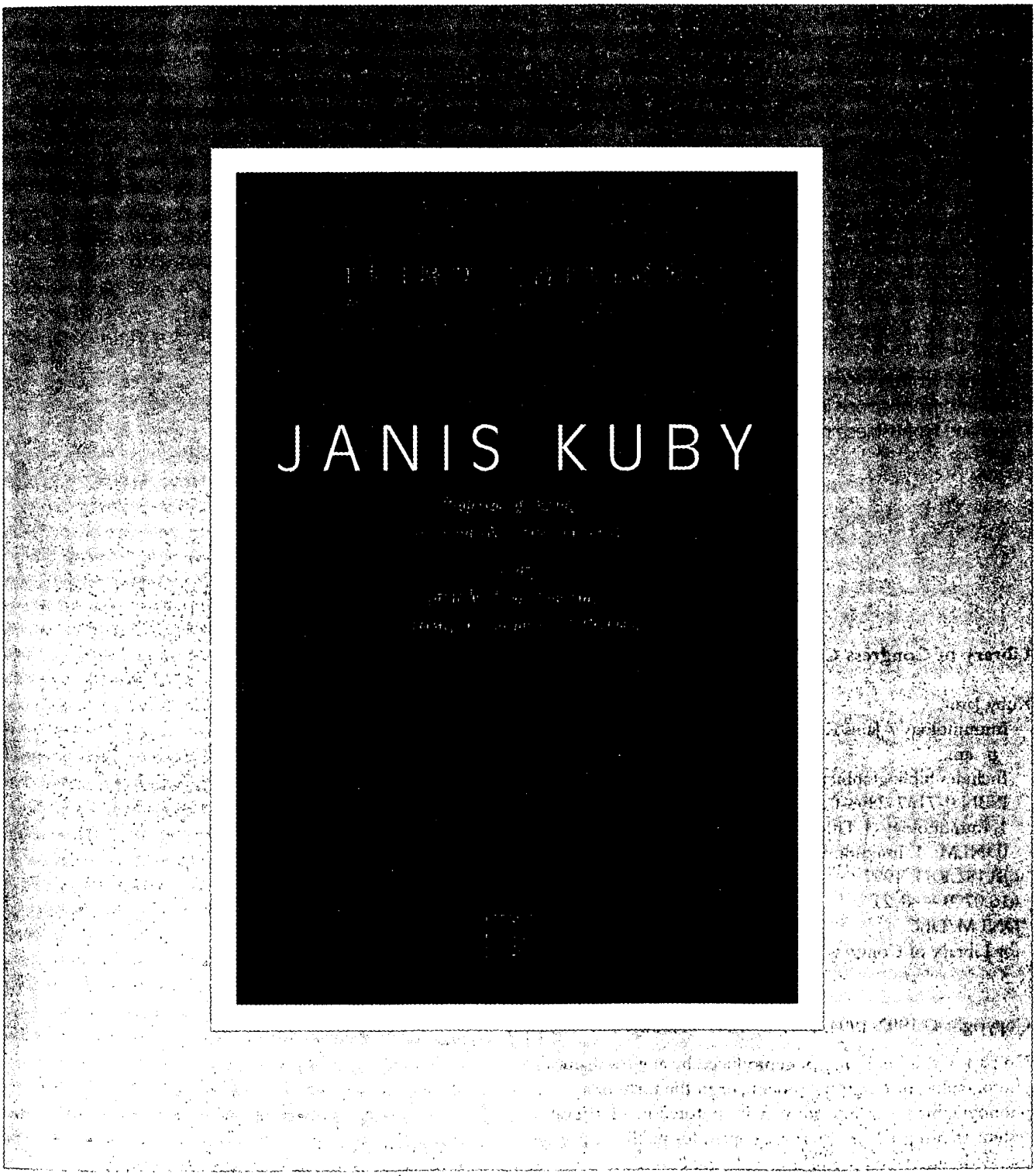
Double Immunodiffusion (Ouchterlony Method)

In the Ouchterlony method both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equivalence is reached, a visible line of precipitation forms (see Figure 6-6). This simple technique is an effective *qualitative* tool for determining the relationship between antigens and the number of different Ag-Ab systems present.

The pattern of the precipitin lines that form when two different antigen preparations are placed in adjacent wells indicates whether or not they share epitopes (Figure 6-8):

- **Identity** occurs when two antigens share identical epitopes. The antiserum forms a single precipitin line with each antigen that grow toward each other and fuse to give a single curved line of identity.
- **Nonidentity** occurs when two antigens are unrelated (i.e., share no common epitopes). The antiserum forms independent precipitin lines that cross.
- **Partial identity** occurs when two antigens share some epitopes but one or the other has a unique epitope(s). The antiserum forms a line of identity with the common epitope(s) and a curved spur with the unique epitope(s).

IMMUNOLOGY



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ABOUT THE COVER AND FRONTISPIECE

Interactions of cell adhesion molecules, with different ones involved at different times, are responsible for recruiting leukocytes to inflammatory sites and for their migration through the vascular endothelium. Slowed by vasodilation, leukocytes drift against vessel walls, where selectins are responsible for a loose adherence known as "rolling." This initial step in leukocyte migration is shown in a false-color scanning electron micrograph. (See Chapter 15 for more information.)

Library of Congress Cataloging-in-Publication Data

Kuby, Janis.
Immunology / Janis Kuby. — 3rd ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-7167-2868-0
1. Immunology. I. Title.
[DNLM: 1. Immune System. 2. Immunity. QW 504 K95i 1997]
QR181.K83 1997
616.07'9 — dc21
DNLM/DLC
for Library of Congress 96-52442
CIP

Printed in the United States of America

Third printing, 1998